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ISOLATION AND CHARACTERIZATION OF *FUSARIUM OXYSPORUM* F.SP. *VASINFECTUM* CAUSATIVE AGENT OF COTTON WILT DISEASE IN PUNJAB, PAKISTAN

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A B S T R A C T

Fusarium cotton wilt (FW) is one of the most economically devastating cotton diseases worldwide and the threatening agent of this disease is *Fusarium oxysporum* f.sp. *vasinfectum* (FOV). This study aimed to add more information towards the isolation and identification of *F. oxysporum* f. sp. *vasinfectum* (*FOV*), which could probably be responsible for the wilting of their corresponding host, *Gossypium hirsutum*. Roots samples were collected from plants showing typical symptoms of wilting from three different regions (Kasur, Faisalabad, Multan) of province Punjab, Pakistan. For further analysis, samples were immediately transported to the microbiology laboratory of Government College University Faisalabad, Pakistan (GCUF). After surface sterilization of root, each cut piece (5 mm) of the root was inoculated on Potato Dextrose Agar (PDA) media and incubated at 25°C±2 °C for 7 days. The different obtained co1lonies were identified using the standard microbiological techniques such as macro-morphological, microscopic, and Polymerase Chain Reaction (PCR). The pathogenicity test was also performed in the greenhouse for confirmation of Koch's postulate. This test showed that plants infected with FOV start wilting and yellowing of leaves which confirmed that FOV was linked with cotton wilt and responsible for significant economic loss. Good agricultural practices and proper management of *FOV* will permit the production of healthy seedlings and subsequently aid in the improvement in cotton yield.

Keywords: *Fusarium oxysporum*, Wilt Pathogen, Microscopy, PCR.

INTRODUCTION

Fusarium oxysporum is the most common cosmopolitan soil-borne pathogen that known for their ability to cause wilt disease in a wide variety of plant species such as cotton, tomato, and chickpea and cause severe damage and yield losses (Constantin *et al*., 2019; El-Hassan and Gowen, 2006; Gopalakrishnan *et al.,* 2011; Van Der Does *et al.,* 2008). *Fusarium* has several formal species (f.sp.) like *Fusarium oxysporum* f.sp. *cubense (Foc)* and *F. oxysporum* f.sp. *vasinfectum (FOV)* affecting specific hosts such as in banana and cotton *(Gossypium hirsutum L.)* respectively. *Fusarium oxysporum* f.sp. *vasinfectum* (*FOV*) is an important disease affecting agents, nearly all cotton-growing regions of the world, and reduced cotton yield to intolerable levels (Seo *et al.,* 2020). Many other species of *Fusarium* are also linked with roots of cotton but their role as pathogen is still not clear (Asif *et al*., 2023). *FOV* morphologically identical pathogenic, nonpathogenic and even beneficial strains. In Pakistan *F. oxysporum* f. sp. *vasinfectum* has been reported as a pathogen of the largest Kharif cotton crop which is grown on 12% of total cultivated area and causing serious economic loss under favorable environmental conditions (Rajput *et al.,* 2006).

F. oxysporum is an ascomycetes widespread pathogen with a parasitic phase in the plant and can also persist in the soil or debris as a saprophytic phase for many years in dormant form producing chlamydospores (Bennett, 2012; Zhang *et al.,* 1996). The hyphae of

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these vascular fungi can enter the root by penetrating directly through the cortical root cells or via wounds (Agbaglo *et al.,* 2020; Hillocks *et al.,* 2000). Moreover, *F. oxysporum* f. sp. *vasinfectum* (*FOV*) can be transmitted by contaminated shoes, knives, vehicles, and infected seeds (Hillocks *et al.,* 2002). After the invasion, *F. oxysporum* f. sp. *vasinfectum* (*FOV*) enters the vascular tissues of plants and subsequently colonizes in the xylem. In an attempt to limit vessel colonization the host induces different defense responses, which include the production of gels, gums, and tyloses (Pietro *et al.,* 2003). These compounds block the vessels, thereby compromising the plant's ability to transport water, result induce the typical wilting symptoms such as severe foliage wilting, drying, withering of older leaves, stunting of plants, discoloration (orange to brown) of the plant crown, and eventually plants become malformed and die (Fang *et al.,* 2012; Halpern *et al.,* 2018).

F. oxysporum f. sp. *vasinfectum* is the most destructive and still uncontrollable vascular pathogen of cotton that can infect plants at any stage of growth, and found to be more lethal in the cold environment at a mean temperature of about 23°C (Cianchetta *et al.,* 2015). Therefore, the long moon soon season of Pakistan is more prone for this pathogen. *F. oxysporum* f. sp. *vasinfectum* can persist in the soil for many years by making chlamydospores or conidia. Therefore, it is difficult to completely eradicated *F. oxysporum* f. sp. *vasinfectum* from the soil (Davis *et al.,* 2006; Lamia *et al.,* 2017; Merrouche *et al.,* 2017). Currently, there are no effective strategy available for control of *Fusarium* disease. However, integrated management practices like resistant cultivars, cultural technique, chemical control, crop rotation, and biocontrol agents are the most effective strategies used to control *F. oxysporum* f.sp. *vasinfectum* and every technique has its own value (Asif *et al.,* 2020; Jiménez *et al.,* 2011; Maitlo *et al*., 2014).

F. oxysporum f. sp. *vasinfectum* is one of the most prevalent and notorious pathogens of cotton plants causing massive damages in open fields and even in the greenhouse. This incite the urgency to identify the fungus to better understand and develop proper control measures. In spite of legitimacy, most commonly preferred method used to identify *Fusarium oxysporum* are macro and microscopic features. But these methods are time consuming and limited accuracy. Therefore, recently PCR amplification method based on the nucleotide sequence getting attention due to maximum validity *(Singha et al.,* 2016). Therefore, the objective of this study was isolation and identification of fungus associated with cotton wilt, which is one of the main economic crops of Pakistan, and verifies its pathogenicity using Koch's postulate and endorse adequate control measure.

MATERIALS AND METHODS

Study area: The whole study was performed at the Department of Microbiology, Government College University Faisalabad, Pakistan (GCUF), and the Cotton Research Station, Ayub Agricultural Research Institute Faisalabad, Pakistan.

Sample collection: From each field of cotton plants, two to three roots samples at various stages of plant growth were collected characterized with visible wilt symptoms. Root samples were shipped overnight to the Laboratory of Microbiology Department of GCUF, and stored at 4°C until further processed. A total of 300 samples were collected from fields of three districts; Kasur, Multan, and Faisalabad of province Punjab, Pakistan. Necessary information like sampling date, location, row spacing, and cotton growth stage were mentioned on these paper bags.

Isolation of fungal pathogen: For the isolation of *Fusarium*, root sections were rinsed with tap water to remove dust particles and soaked in 1% sodium hypochlorite for surface sterilization. After surface sterilization, samples were again washed twice with distilled water to remove toxic effect of sodium hypochlorite. After that, roots were cut into 1-2 cm long sections by using sterilized scissors. These air-dried sections were inoculated to Petri plates containing Potato Dextrose Agar (PDA) media (Difco, USA). The PDA growth medium was supplemented with streptomycin sulfate (300 mg/L) and incubated for 7 days at 25ºC±2ºC. After visible mycelial growth, the hyphal tips from the advancing mycelium were taken and transferred into the culture slants containing PDA medium for purification, identification, and maintenance of pure culture. For sub-culture fresh plates of PDA were prepared, distinct fungi colonies from primary cultures were cut out using a sterile scalpel and then transferred to the fresh PDA plates to obtain pure cultures. Inoculated plates were sealed with parafilm wax and incubated at

room temperature 25 °C±2 °C in the dark for 7 days. To obtain the pure culture of *F. oxysporum* f. sp. *vasinfectum*, a single colony was picked and cultured again and again using a single spore culture technique unless attainment of single pure culture. Morphological identification was performed according to Leslie and Summerell (2006) .

Characterization and identification of the filamentous fungus: Different standard microbiological techniques like macro-morphological, microscopic, and polymerase chain reactions were used for the identification of pure cultures of the pathogen fungi *F. oxysporum* f. sp. *vasinfectum*.

Macro-morphological characterization: For macroscopic observation, the cultural appearances (colony texture, color, reverse plate texture, margin, form, elevation, and hyphae) were observed on PDA. Colony color was confirmed using the Methuen handbook of the color chart (Kornerup *et al.,* 1978). A laboratory manual and a pictorial atlas for identification of fungi by Watanabe (2010) were equally used in the description of colony morphology (Ghosh *et al.,* 2018).

Microscopic morphology identification: The microscopic features of the fungus isolate such as spore type (microconidia, macroconidia, chlamydospores), spore length, septum, and hyphal type were observed through slide culture test and lactophenol cotton blue staining method (Mailafia *et al.,* 2017)

Lactophenol cotton blue staining: Lactophenol cotton blue solution was prepared. A fragment of a fungal isolate was placed on a grease-free clean slide using a sterile inoculating needle. The fragment of a fungal isolate was finely spread on the glass slide with a teasing needle. lactophenol cotton blue solution (2-3 drops) was put on the glass slide and then left for a few seconds to completely absorb and spread the stain on mycelium. To eliminate air bubbles, we gently placed a coverslip on mycelium with little pressure. Then the slide was examined under the light microscope to record the type of spore, hyphae, and other special structures using an objective lens first with 40X and then with 100X. The isolates were identified following the description of Oyeleke and Manga (2008).

Slide culture test: Put a small piece of potato dextrose agar with a sterile inoculating loop on a clean, sterile, grease-free glass slide. Then a fragment of a mycelium was placed on the slide containing a piece of PDA with an inoculating needle and a coverslip was placed in such a way to allow aeration. Then the slide was incubated for seven days at 25°C ±2°C in a closed sterile petri dish, a sterile soaked filter paper was placed in the bottom of the petri dish for moisture. Afterwards, stained with 1-2 drops lactophenol cotton blue dye. Light microscopy was used to detect spore, hyphae and other special structures using the objective lens, first with 40X and then 100X. (Samuel *et al.,* 2016).

Identification by PCR: Extraction of DNA was performed using the Genomic DNA Purification Kit (Thermo Scientific ®) according to the manufacturer's instructions from isolated FOV. A total volume of 25µL of PCR mixture was carried out for target gene amplification using universal primers (ITS1 forward TCCGTAGGTGAACCTGCGG; ITS4 reverse TCCTCCGCTTATTGATATGC) studied by (Moricca *et al.,* 1998) following the condition of 94ºC for 1min., 50ºC for 1min. and a final extension at 72ºC for 3min.

Pathogenicity test: A pathogenicity test was carried out to confirm isolated fungi that were responsible for the spoilage of cotton plants. For this purpose, a pot experiment was carried out for the pathogenicity test. Fresh and disease free cotton seeds were soaked for 3 mint in H2SO4 for surface sterilization. Then rinsed thoroughly three time with distilled water and sown in autoclave sterilized soil. At the 3-4 leaflets stage, a 5 mm mycelial plug of 7 days old pathogen culture was inoculated at two opposite corners of the pot. The disease symptoms were recorded after 30 days of inoculation. Uninoculated (UI) control was also maintained. The plants were watered regularly on alternate days to keep plant healthy (Aslam *et al.,* 2019). The fungi were later re-isolated from the infected cotton plants and compared with the initial isolates.

RESULTS

Isolation of *F. oxysporum* f.sp *vasinfectum:* In the current study, total 300 samples were collected from various fields of selected district of Punjab, Pakistan.

Morphological identification: After 7 days of incubation at a temperature of 25 ± 2°C, the isolated pathogen show white cotton appearance with fluffy growth which turn pink or purple on the back of the Petri plate containing with Potato Dextrose Agar (PDA) medium (Figure 1). The colony diameter of the pathogen ranged from 8-8.5 cm. Macro-morphological study exhibited that *F. oxysporum* f.sp *vasinfectum* showed highly variable growth on PDA medium with delicate, slightly lobed or smooth , margins.

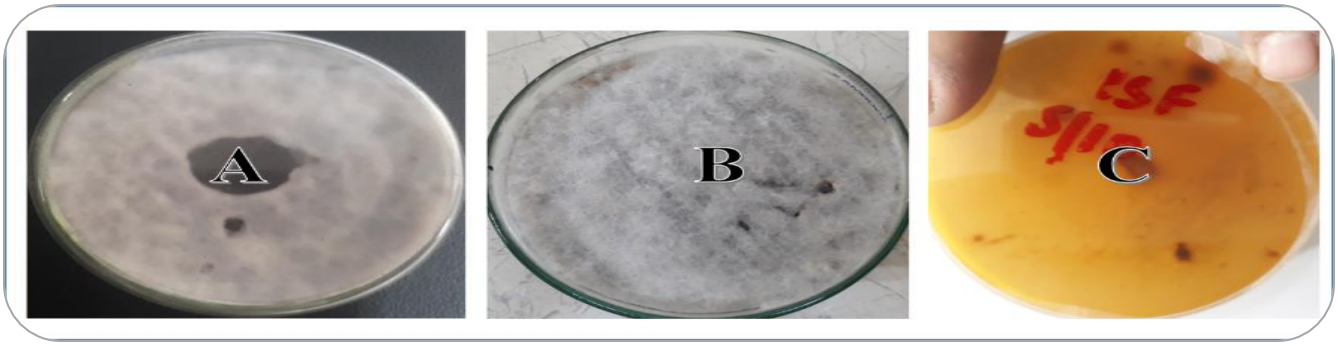

Figure 1. Colony morphology of FOV on PDA after 7 days of incubation at 25°C±2 °C. Whitish cottony appearance from the front view of Petri plate (A&B), while yellowish appearance from the reverse view of Petri plate (C).

Microscopic identification: The microscopic examination of *F. oxysporum* f.sp *vasinfectum* under light microscope at 100X revealed three kinds of spores. Numerous micro and macroconidia were observed on PDA. Microconidia were produced on short monophialides singly, with zero to one septate and oval to renal shaped (Figure 2A). The microconidia size ranged from 13.2-4.3 and 2.50-4.50 micrometers. Macroconidia were crescent-shaped, large spores with size range 20.27-40.50 and 5.00-6.75 µm. Macroconidia usually divided into three to five different cells, falcate to almost straight, thin-walled, both ends almost pointed and in some cases slightly curved (Figure 2B). Macroconidia were produced in sporodochia as well as on normal hyphae. The Chlamydospores appeared in the spherical or elliptical form on a 3-6 week-old fungus culture. They produced in terminal or intercalary chains with size ranged 18.7-18.2 x 20.3-18.9 µm (Figure 2C).

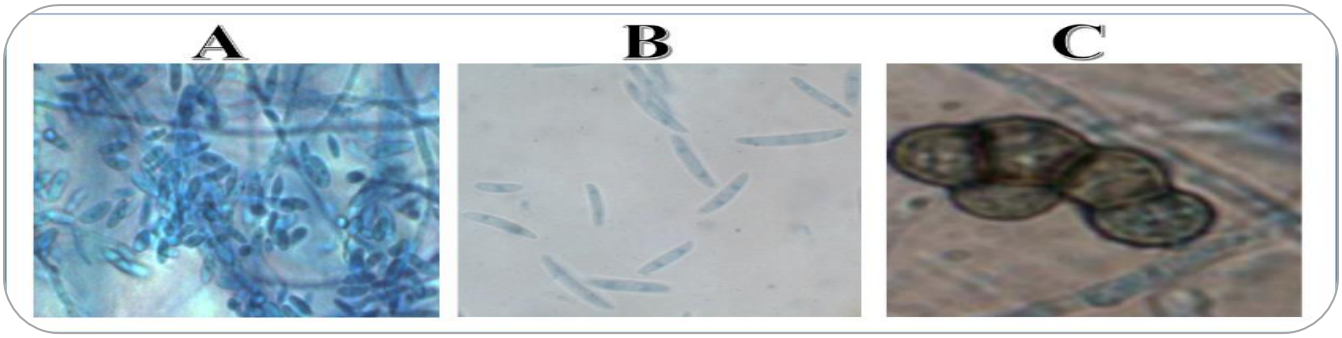

Figure 2. Micro-morphological presentation of FOV after 7 days of incubation at 25°C±2°C on PDA showed 3 kinds of spores (A) Microconidia (B) Macroconidia (C) Chlamydospores

PCR detection: PCR analysis was conducted for the proper identification of the *F. oxysporum*. The internal transcribed spacer (ITS) region of the *Fusarium* isolate was amplified using universal primers ITS1 and ITS4. After agarose gel electrophoresis of the PCR amplified DNA, it was observed that the selected primer pair exclusively amplified the expected 18S rDNA band of 453 bp size and results demonstrated that in test samples afore-mentioned gene was dramatically amplified which validated its purification and identification (Figure 3).

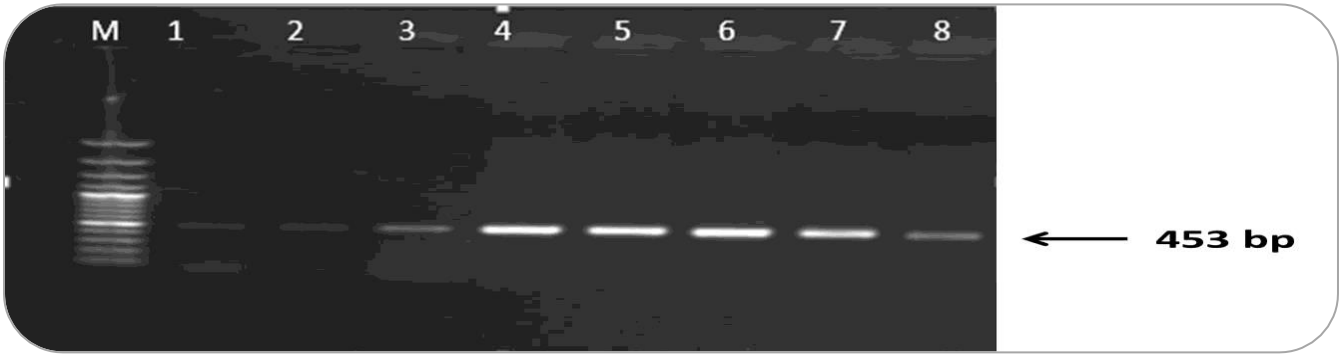

Figure 3. PCR using universal primers ITS1 and ITS4. M line indicates the molecular weight marker, Lane 8 is a positive control, and Lane 1, 2, 4, 5, 6, 7 are samples. 453 base pair is the target gene. Samples were run on 1.5% agarose gel.

Pathogenicity test: During pot assay, Fusarium wilt infected plants exhibited yellowing, drying, and drooping of leaves and plants showed stunted growth (Figure 4A) compared to UI Control plant labeled as Healthy plant (Figure 4B). As the disease progressed, the plant exhibited drying and wilting on the 20th day after inoculation. The re-isolation fungus from the roots of wilted cotton plants confirmed that it is the same starting strains that were used to prepare initial inoculums and allowed us to establish a causative relationship between isolates and the disease.

Figure 4. Pathogenicity assay *in-vivo* models: (A); In pot assay, healthy uninoculated (UI) plant was used as control (B); whereas, sterilized cotton seeds were sown in sterilized soil and 7 mm mycelial plug of the FOV was given at 3-4 leaflets stage, showed the wilt symptoms and also labeled as an infected plant.

DISCUSSION

Cotton is economically a major crop and considers as a lifeline role in the world economy. Pakistan is the 4th largest producer of cotton and nearly 60 % of its overseas earnings were produced from cotton products. The most cotton grown area in Pakistan are Province Punjab and Sindh. Like other major crops, pest contributes 10-30 % annual cotton loss worldwide (Tarazi *et al.,* 2020). There are more than 60 diseases that reduce the yield of cotton but Fusarium wilt is one of the most important diseases. *F. oxysporum* has been found as a notorious pathogen of Fusarium cotton wilt worldwide. In the current study, fungus isolated and identified from roots of wilted cotton plants, suggesting that *F. oxysporum* f.sp *vasinfectum* is a causative agent of cotton wilt and responsible for the significant loss of cotton yield. These findings were congruent with many previous studies also reported that *F. oxysporum* f.sp *vasinfectum* is accountable for fusarium cotton wilt (Da Silva *et al.,* 2019; Li *et al.,* 2017; Okungbowa and Shittu 2012; Zhu *et al.,* 2020). *F. oxysporum* f.sp *vasinfectum* invades the roots and stems tissues, blocking the water-conducting channels and causing leaves to turn yellow, dry, and eventually plant (Farias *et al*., 2019).

In current study macro-morphological study showed white cotton appearance with fluffy growth of *F. oxysporum* f.sp *vasinfectum* on PDA medium. While pink or purple colour was observed on the reverse side of the Petri plate. *F. oxysporum* f.sp *vasinfectum* showed highly variable growth on PDA medium (Mohammed *et al.,* 2016). The colony diameter of the pathogen ranged from 8-8.5 cm on the seven days of inoculation in PDA medium. Isolates from different areas slightly showed variation in their growth which is compatible with one previous study. All these features also corresponded to the critical morphological features of *F. oxysporum* species cited by Leslie and Summerell (2006). Amini and Sidovich (2010) reported white cottony to pink often with purple color of mycelium.

The observations of *F. oxysporum* f.sp *vasinfectum* under light microscope at 100X revealed three kinds of spores. Microconidia with mean size ranged from 13.2-4.3 and 2.50 - 4.50 micrometers with zero to one septate and oval to renal shaped. Macroconidia were crescent-shaped, large spores, both ends almost pointed with 3-5 septate and size range 20.27 - 40.50 and 5.00 - 6.75 µm. The Chlamydospores appeared in the spherical or elliptical form with size ranged 18.7-18.2 x 20.3-18.9 µm. These finding also compatible with (AL-Taae *et al*., 2019; Amini and Sidovich 2010) who proved microconidia were oval shaped with zero septate and macroconidia were curve or straight with 3-5 septate. Chlamydospore was smooth and rough walled and no sexual stage was observed in isolates. Altaf *et al.* (2014) also highlighted that micro-conidia were oval shaped with 2 cells. These findings were also seemed to be similar with previous research and characteristics correspond to *Fusarium oxysporum* (Summerell *et al.,* 2003).

Sometimes, it was not easy to discriminate *Fusarium* species on the base of microscopic or morphological

features (Gupta *et al.,* 2010). Therefore, PCR analysis was conducted for the proper identification of the *F. oxysporum*. The internal transcribed spacer (ITS) region of the *Fusarium* isolate was amplified using universal primers ITS1 and ITS4. After agarose gel electrophoresis of the PCR amplified DNA, it was observed that the selected primer pair exclusively amplified the expected 18S rDNA band of 453 bp size and results demonstrated that in test samples afore-mentioned gene was dramatically amplified compared to the negative control which validated its purification and identification. Morrica *et al.* (1998) also identified the *F. oxysporum* f.sp. *vasinfectum* by PCR. (AL-Taae *et al*. (2019) also used a universal primer (ITS1 & ITS4) and identified *F. oxysporum* by PCR method.

In pathogenicity assay, *F. oxysporum f.sp. vasinfectum* showed wilt symptoms after inoculation like drying and drooping of leaves with stunted growth compared to UI Control plant. The re-isolation of fungus from roots of wilted cotton plants confirmed the causative relationship between isolate and the disease. These finding proved Koch's postulates. These outcomes are compatible with numerous previous studies (Al-Taae *et al.,* 2019; Berruezo *et al.,* 2018; Poletto *et al.,* 2020). Zhu *et al.* (2020) also performed pathogenicity test of *F. oxysporum* and found it virulence against its corresponding host. Amini and Sidovich (2010) also carried out pathogenicity test of *F. oxysporum* and appeared symptoms were recorded after 15 days of inoculation.

CONCLUSION

From the findings of this study, it is concluded that *F. oxysporum f.sp. vasinfectum* is a pathogen of cotton wilt and responsible for huge economic loss worldwide. Early detection of FOV will help proper management and permit the production of healthy seedlings. The healthy seedlings will guarantee a large-scale production of cotton.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this study.

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REFERENCES

Agbaglo, S.Y., S.T. Nyaku, H.D. Vigbedor and E.W. Cornelius. 2020. Pathogenicity of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on growth and yield of two okra varieties cultivated in ghana. International Journal of Agronomy, 12: 1-12.

AL-Taae, W. and A.K. Al-Taae. 2019. First molecular identification of *Fusarium oxysporum* causing Fusarium wilt of Armenian cucumber in Iraq, IOP Conference Series: Earth and Environmental Science. IOP Publishing, Bristol, UK, 338: 1-6.

Altaf, R., C.A. Rauf, F. Naz and G. Shabbir. 2014. Surveillance and morphological characterization of Fusarium isolates associated with lentil wilt. Pakistan Journal of Phytopathology, 26: 85-90.

Amini, J. and D. Sidovich. 2010. The effects of fungicides on *Fusarium oxysporum* f.sp. *lycopersici* associated with Fusarium wilt of tomato. Journal of Plant Protection Research, 50: 173-178.

Asif, R., M. H. Siddique, S. Hayat, I. Rasul, H. Nadeem, M. Faisal and S. Muzammil. 2023. Efficacy of *Saccharothrix algeriensis* NRRL B-24137 to suppress *Fusarium oxysporum f. sp. vasinfectum* induced wilt disease in cotton. PeerJ, 11: e14754.

Asif, R., M.H. Siddique, S.A. Zakki, M.H. Rasool, M. Waseem and S. Muzammil. 2020. *Saccharothrix Algeriensis* NRRL B-24137 potentiates chemical fungicide carbendazim in treating *Fusarium Oxysporum* f. sp. *Vasinfectum*-induced cotton wilt disease. Dose-Response, 18: 15593.

Aslam, S., M.U. Ghazanfar, N. Muni and M.I. Hamid. 2019. Managing Fusarium wilt of pea by utilizing different application methods of fungicides. Pakistan Journal of Phytopathology, 31: 81-88.

Bennett, R. 2012. Survival of *Fusarium oxysporum* f.sp. *vasinfectum* chlamydospores under solarization temperatures. Plant Disease, 96: 1564-1568.

Berruezo, L.A., G.E.M. Cárdenas, Ed.M. Harries, S.A. Stenglein, R.N. Curti, M.S. Rodriguero and M.Z. Galván. 2018. Characterization of *Fusarium* species associated with tobacco diseases in Northwestern Argentina. European Journal of Plant Pathology, 151: 1065-1079.

Cianchetta, A.N. and R. Davis. 2015. Fusarium wilt of cotton: management strategies. Crop Protection, 73: 40-44.

Constantin, M.E., F.J. Lamo, B.V. Vlieger, M. Rep and F.L. Takken. 2019. Endophyte-mediated resistance in tomato to *Fusarium oxysporum is* independent of ET, JA, and SA. Frontiers in Plant Science, 10: 1-14.

Da-Silva, M.B., R.F. Davis, H.K. Doan, R.L. Nichols, R.C.

Kemerait, H.C. Halpern, M.T. Brewer, G. Jagdale and P.E. Chee. 2019. Fusarium wilt of cotton may commonly result from the interaction of *Fusarium oxysporum* f. sp. *vasinfectum* with *Belonolaimus longicaudatus*. Journal of Nematology, 51: e201915

Davis, R. M., P. D. Colyer, C. S. Rothrock and J. K. Kochman. 2006. Fusarium wilt of cotton: population diversity and implications for management. Plant Disease, 90: 692-703.

El-Hassan, S. and S. Gowen. 2006. Formulation and delivery of the bacterial antagonist *Bacillus subtilis* for management of lentil vascular wilt caused by *Fusarium oxysporum* f.sp. *lentis*. Journal of Phytopathology, 154: 148-155.

Fang, X., M.P. You and M.J. Barbetti. 2012. Reduced severity and impact of Fusarium wilt on strawberry by manipulation of soil pH, soil organic amendments and crop rotation. European Journal of Plant Pathology, 134: 619-629.

Farias, O.R., L.C. Nascimento, J.M.F. Lima, H.A.O. Silva, M.D. Mello, L.A.Bruno and N.H.C. Arriel. 2019. Biocontrol potential of *Trichoderma* and *Bacillus* species on *Fusarium oxysporum* f.sp *vasinfectum*. Journal of Experimental Agriculture International, 34: 1-11.

Ghosh, S.K., S. Banerjee, S. Pal and N. Chakraborty. 2018. Encountering epidemic effects of leaf spot disease (*Alternaria brassicae*) on Aloe vera by fungal biocontrol agents in agrifields, An ecofriendly approach. PloS One, 13: 0193720.

Gopalakrishnan, S., S. Pande, M. Sharma, P. Humayun, B.K. Kiran, D. Sandeep, M.S. Vidya, K. Deepthi and O. Rupela. 2011. Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of Fusarium wilt of chickpea. Crop Protection, 3: 1070-1078.

Gupta. S., D. Chakraborti, A. Sengupta, D. Basu and S. Das. 2010. Primary metabolism of chickpea is the initial target of wound inducing early sensed *Fusarium oxysporum* f.sp. *ciceri* race. Plos One, 5: e9030.

Halpern, H., A. Bell, T. Wagner, J. Liu, R. Nichols, J. Olvey, J. Woodward, S. Sanogo, C. Jones and C. Chan. 2018. First report of Fusarium wilt of cotton caused by *Fusarium oxysporum* f. sp. *vasinfectum* race 4 in Texas, USA. Plant Disease, 102: 446-446.

Hillocks, R., E. Minja, A. Mwaga, M.S. Nahdy and P. Subrahmanyam. 2000. Diseases and pests of pigeonpea in eastern Africa: a review. International Journal of Pest Management, 46: 7-18.

Hillocks, R.A. and T. Kibani. 2002. Factors affecting the distribution, incidence and spread of Fusarium wilt of cotton in Tanzania. Experimental Agriculture, 38: 13-27.

Jiménez Díaz, R.M. and M.M. Jiménez-Gasco. 2011. Integrated management of Fusarium wilt diseases. Control of Fusarium Diseases, 2011: 177-215.

Kornerup, A., J. Wanscher. 1987. Methuen Colour Handbook. Methuen London Ltd., London.

Lamia, B., B. Messaoud, C. Ahmed and K. Lakhdar. 2017. Activity of plant growth promoting rhizobacteria (PGPRs) in the biocontrol of tomato Fusarium wilt. Plant Protection Science, 53: 78-84.

Leslie, J. and B. Summerell. 2006. Fusarium laboratory workshops--A recent history. Mycotoxin Research, 22: 73-74.

Li, X., Yn. Zhang, C. Ding, W. Xu and X. Wang. 2017. Temporal patterns of cotton Fusarium and Verticillium wilt in *Jiangsu coastal* areas of China. Scientific Reports, 7: 1-8.

Mailafia, S., H.O. God'spower, K. Olabode and R. Osanupin. 2017. Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. Veterinary World, 10: 393-397.

Maitlo, S., R. Syed, M. Rustamani, R. Khuhro and A. Lodhi. 2014. Comparative efficacy of different fungicides against fusarium wilt of chickpea (*Cicer arietinum* L.). Pakistan Journal of Botany, 46: 2305-2312.

Merrouche, R., A. Yekkour, L. Lamari, A. Zitouni, F. Mathieu and N. Sabaou. 2017. Efficiency of *Saccharothrix algeriensis* NRRL B-24137 and its produced antifungal dithiolopyrrolones compounds to suppress *Fusarium oxysporum*-induced wilt disease occurring in some cultivated crops. Arabian Journal for Science and Engineering, 42: 2321-2327.

Mohammed, A.S, N.H. Kadar, M. Kihal, J.E. Henni, J. Sanchez, E. Gallego, J. Garrido and E. Antonio. 2016. Characterization of *Fusarium oxysporum* isolates from tomato plants in Algeria. African Journal of Microbiology Research, 10: 1156-1163.

Moricca, S., A. Ragazzi, T. Kasuga and K. Mitchelson. 1998. Detection of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton tissue by polymerase chain reaction. Plant Pathology, 47: 486-494.

Okungbowa, F. and H. Shittu. 2012. Fusarium wilts: An overview. Environmental Research Journal, 6: 83-102.

Oyeleke, S.B. and S. Manga. 2008. Essentials of laboratory practicals in microbiology. Tobest Publishers, Minna, Nigeria. PP. 36-75.

Pietro, A.D., M.P. Madrid, Z. Caracuel, J. Delgado-Jarana and M.I.G. Roncero. 2003. *Fusarium oxysporum:* exploring the molecular arsenal of a vascular wilt fungus. Molecular Plant Pathology, 4: 315-325.

Poletto, T., M.F.B. Muniz, V.S. Fantinel, R. Harakava and J.M. Rolim. 2020. Characterization and Pathogenicity of *Fusarium oxysporum* associated with *Carya illinoinensis* seedlings. Floresta e Ambiente, 27: 1-9.

Rajput, A.Q., M. Arain, M. Pathan, M. Jiskani and A. Lodhi. 2006. Efficacy of different fungicides against Fusarium wilt of cotton caused by *Fusarium oxysporum* f.sp. *vasinfectum*. Pakistan Journal of Botany, 38: 875-871.

Samuel, O., N. Ogonna and O. Ifeanyi. 2016. Isolation, characterization and identification of microorganisms from spoilt carrots obtained from Ose Market Onitsha. Nigeria Universal Journal of Biomedical Engineering, 4: 6-9.

Seo, S., A. Pokhrel and J.J. Coleman. 2020. The Genome sequence of five genotypes of *Fusarium oxysporum* f. sp. *vasinfectum*: A resource for studies on Fusarium wilt of cotton. Molecular Plant-Microbe Interaction, 33: 138-140.

Singha, I. M., Y. Kakoty, B.G. Unni, J. Das and M.C. Kalita. 2016. Identification and characterization of Fusarium sp. using ITS and RAPD causing fusarium wilt of tomato isolated from Assam, North East India. Journal of Genetic Engineering and Biotechnology, 14: 99-105.

Summerell, B.A., B. Salleh and J.F. Leslie. 2003. A utilitarian approach to Fusarium identification. Plant Disease, 87: 117-128.

Tarazi, R., J.L.S. Jimenez and M.F. Vaslin. 2020. Biotechnological solutions for major cotton (*Gossypium hirsutum*) pathogens and pests. Biotechnology Research and Innovation, 3: 19-26.

Van Der Does, H.C., B. Lievens, L. Claes, P.M. Houterman, B.J. Cornelissen and M. Rep. 2008. The presence of a virulence locus discriminates *Fusarium oxysporum* isolates causing tomato wilt from other isolates. Environmental microbiology, 10: 1475-1485.

Watanabe, T. 2010. Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC press, Boca Raton, Florida, United States.

Zhang, J., C. Howell, J. Starr and M. Wheeler. 1996. Frequency of isolation and the pathogenicity of *Fusarium* species associated with roots of healthy cotton seedlings. Mycological Research, 100: 747-752.

Zhu, Y., P. Lujan, T. Wedegaertner, R. Nichols, A. Abdelraheem, J. Zhang and S. Sanogo. 2020. First report of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 causing Fusarium wilt of cotton in New Mexico, USA. Plant Disease, 104: 588-588.

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